

Applicant : Jei-Fu Shaw et al.
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Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently amended) An isolated nucleic acid comprising a mutant DNA encoding a ~~*Candida rugosa* lipase~~ wild-type *Candida rugosa* lipase or a *Candida rugosa* variant, wherein the mutant DNA is at least 80% identical to a wild-type DNA encoding the *Candida rugosa* lipase, and includes at least 12 codons substituted for CTG codons in the wild-type DNA, each of the 12 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.
2. (Original) The nucleic acid of claim 1, wherein at least 15 codons correspond to CTG codons in the wild-type DNA, each of the 15 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.
3. (Currently amended) The nucleic acid of claim 2, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.
4. (Original) The nucleic acid of claim 2, wherein all codons correspond to CTG codons in the wild-type DNA, each of the codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.
5. (Currently amended) The nucleic acid of claim 4, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.
6. (Currently amended) The nucleic acid of claim 5, wherein the amino acid sequence of the *Candida rugosa* lipase variant is SEQ ID NO:4.

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7. (Currently amended) The nucleic acid of claim 1, wherein the mutant DNA is at least 85% identical to [[a]] the wild-type DNA encoding the *Candida rugosa* lipase.

8. (Original) The nucleic acid of claim 7, wherein at least 15 codons correspond to CTG codons in the wild-type DNA, each of the 15 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

9. (Currently amended) The nucleic acid of claim 8, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

10. (Original) The nucleic acid of claim 8, wherein all codons correspond to CTG codons in the wild-type DNA, each of the codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

11. (Currently amended) The nucleic acid of claim 10, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

12. (Currently amended) The nucleic acid of claim 11, wherein the amino acid sequence of the *Candida rugosa* lipase variant is SEQ ID NO:4.

13. (Currently amended) The nucleic acid of claim 1, wherein the mutant DNA is at least 90% identical to [[a]] the wild-type DNA encoding the *Candida rugosa* lipase.

14. (Original) The nucleic acid of claim 13, wherein at least 15 codons correspond to CTG codons in the wild-type DNA, each of the 15 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

15. (Currently amended) The nucleic acid of claim 14, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

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16. (Original) The nucleic acid of claim 14, wherein all codons correspond to CTG codons in the wild-type DNA, each of the codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

17. (Currently amended) The nucleic acid of claim 16, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

18. (Currently amended) The nucleic acid of claim 17, wherein the amino acid sequence of the *Candida rugosa* lipase variant is SEQ ID NO:4.

19. (Previously presented) A microorganism comprising the nucleic acid of claim 1, wherein the microorganism is a bacterium or a yeast.

20. (Previously presented) The microorganism of claim 19, wherein the bacterium is *E. coli* and the yeast is *P. pastoris*.

21. (Currently amended) The microorganism of claim 19, wherein the mutant DNA is at least 85% identical to [[a]] the wild-type DNA encoding the *Candida rugosa* lipase.

22. (Previously presented) The microorganism of claim 21, wherein the bacterium is *E. coli* and the yeast is *P. pastoris*.

23. (Currently amended) The microorganism of claim 19, wherein the mutant DNA is at least 90% identical to [[a]] the wild-type DNA encoding the *Candida rugosa* lipase.

24. (Previously presented) The microorganism of claim 23, wherein the bacterium is *E. coli* and the yeast is *P. pastoris*.

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25. (Previously presented) An isolated nucleic acid, comprising a DNA of SEQ ID NO:3 or a degenerate variant thereof.

26. (Currently amended) A method for preparing a mutant DNA encoding a ~~*Candida rugosa*~~ lipase wild-type *Candida rugosa* lipase or a *Candida rugosa* variant, comprising:
providing a wild-type DNA encoding a *Candida rugosa* lipase; and
conducting PCR amplification by mixing the wild-type DNA, a DNA polymerase, a pair of external primers encompassing the entirety of the wild-type DNA, and a number of pairs of internal primers respectively encompassing fragments of the wild-type DNA, wherein each of the internal primers includes one or more of universal codons and anticodons for serine selected from TCT, TCC, TCA, TCG, AGT, AGC, AGA, GGA, TGA, CGA, ACT, and GCT, in which the universal codons and anitcodons substituted for at least 12 CTG codons in the wild-type DNA; and each internal primer overlaps with another internal or external primer in a manner that a mutant DNA encoding the mutant *Candida rugosa* lipase is obtained.

27. (Original) The method of claim 26, wherein at least 15 codons correspond to CTG codons in the wild-type DNA, each of the 15 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

28. (Currently amended) The method of claim 27, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

29. (Original) The method of claim 27, wherein all codons correspond to CTG codons in the wild-type DNA, each of the codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC.

30. (Currently amended) The method of claim 29, wherein the amino acid sequence of the *Candida rugosa* lipase variant is at least 90% identical to SEQ ID NO:4.

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31. (Currently amended) The method of claim 30, wherein the amino acid sequence of the *Candida rugosa* lipase variant is SEQ ID NO:4.

32. (Previously presented) The method of claim 26, wherein the mutant DNA is SEQ ID NO:3 or a degenerate variant thereof.

33. (Withdrawn) A chimeric *Candida rugosa* lipase comprising a substrate interacting domain of a first *Candida rugosa* lipase and a non-substrate interacting domain of a second *Candida rugosa* lipase.

34. (Withdrawn) The lipase of claim 33, wherein the second *Candida rugosa* lipase is SEQ ID NO:6.

35. (Withdrawn) The lipase of claim 33, wherein the first *Candida rugosa* lipase is SEQ ID NO:2, 4, 8, or 10.